

1 agctactcag gaggctgaga cagagaatc gcttgaaccc gggaggcaga ggttgacgtg
61 agccgagatc acgccactag actccatcca gcctgggcga aagagcaaga ctccgtctca
121 aaaaaaaaaa tcgttacaat ttatggtgga ttactccctt tttttacct catcaagaca
181 cagcactact ttaaagcaaa gtcaatgatt gaaacgcctt tctttcctaa taaaaaggag
241 attcagtcct taagattaat aatgtagtag ttacacttga ttaaagccat cctctgctca
301 aggagaagct ggagaaggca ttctaaggaa aaaggggcag ggttgaaact cggacgcac
361 ccaactgagcc gagacaagat tctgctgtag tcagtctgc ctgggaatct attttcaca
421 agttctccaa aaaatgtgat gatcaaaact aggaattagt gttctgtgtc ttaggcccta
481 aaatcttctt gtgaattcca tttttaagggt agtcgaggtg aaccgcgtct ggtctgcaga
541 ggatagaaaa aaggccctct gatacctcaa gttagtcca cctttaaaga aggtcggaa
601 taaagacgca aagcctttcc cggacgtgcg gaagggaac gtccttccct atggccggaa
661 atggaacttt aatttccctt tcccccaac cagcccgccc gagagagtga ctctcacgag
721 agccgcgaga gtcagcttg gcaatccgtg cggtcgcgg cgctccctt tataagccga
781 ctgcgccggc agcgaccgg gttgcggagg gtgggacctg gaggggtggt ggccattttt
841 tgtctaacc taactgagaa gggcgtagc gccgtgctt tgctccccc gcgctgtttt
901 tctcgtgac tttcagcgg tttcagcgg cggaaaagcc tcggcctgcc gttcattcta
961 gagcaaaaa aaaaatgtcag ctgctggccc gttcgccct cccggggacc tgcggcgggt
1021 cgctgccc gcccgcgaac ccgccttga ggcgcggtc ggccggggc ttctccggag
1081 gcacctactg ccaccgcga gatttggtc tgtcagccg ggtctctcg gggcgaggg
1141 cgagggtcag gcctttcagg ccgcaggaa gactcggctc agagtcccc cgcgcggcgc
1201 gattccctga gctgtgggac gtgcaccag gactcggctc acacatgcag ttcgctttcc
1261 tgttggtggg gggaaacgcg atcgtgcga tcgctcacc ctcgcggca gtgggggct
1321 gtgaaccccc aaacctgact gactgggcca gtgtgctgca aattggcagg agacgtgaag
1381 gcacctccaa agtcggccaa aatgaatgg cagtgaaggc gggttgcctg gagccgttcc
1441 tgcgtgggtt ctccgtctt ccgctttttg ttgccttta tgggtgtatt acaactagt
1501 tcctgctctg cagattttgt tgaggttttt gcttctccca aggtagatct cgaccagtcc
1561 cctcaacggg gtgtgggaga acagtcatct ttttttgaga gatcatttaa cattaatga
1621 atatttaatt agaagatcta aatgaacatt ggaaattgtg ttcccttaac ggtcatcggt
1681 ttatgccaga ggttagaagt tcttttttg aaaaattaga ccttggcgat gacctgagc
1741 agtaggat aacccccaca agctt

Fig. 1

2/29

1 aagcttggac ttgacaaaga aactgcagat catctggacc ccccccccc cccatttagg
 61 ttttaacaatg taccagctat ctgacttaag caaactgtgt tcctcataga taaggcggga
 121 ctgctcatgg tcattgtgaa gttcagttgg gataaacaaa ttttaagggtg cataacaaaa
 181 aacacaaaat gttggtgttt gtttaaaaaa aactaaagaa tttctggagg caggcagtta
 241 cagaaaacat gctgatattc tgagttgcct gctagttggt gccattccac cagagtgaac
 301 acatctctgt tgaccctgat tttctgtagg tctgtctgtg tgtctgtcct ttctccagca
 361 agggctgacc ctaategggg tcccaggacc caagccttga gaaaggcagt agtatgtcat
 421 ctagttgaaa tgacacattc tctacagtgt ccaaatagaca tctttgtgct agacagaaca
 481 ttttattgga tggactatgg ctgaccactt ggcttggggg gggggggaag gggccgcaa
 541 gggcgggggt ccctcatttg cttgttatta acacttgctt gtttgtttac ttgttagtag
 601 gaatctgctc taccacgtgg gttctacatg gttccacagg ggtcacctgg tccgtttttg
 661 ttttctggga cagttttcac aaatgttgct tagactccac gttggctttg aagcctacag
 721 ctatgagcct ctgtgccagt ttatgcagta gtatctctcg ggttgctcct caccgttagt
 781 agtgggtgctc ttagaaggca ccgtgatttt ttgctttcca tctctttccc ctgccatgcc
 841 ttctgtgggt ctctgccagg caccaaactg ttcagaaact ctccagcccg gtagagaacg
 901 gtagggggaa agaactgacg tgtggaaggg atgggcaggg agaagaggca ccgaactcgg
 961 tcttaaacaa aaaaaaaaaa aaaaggagca ttagaaaaaa aaacaaattt gtgacctga
 1021 actacagacc tcctgcctca gcctcctaca agctgggatt ataggctcgg gtcagctacc
 1081 cttgaaatct ttttctttct ggaactcagt acctggttgg ccatgcactc acaagagatc
 1141 cgctgcctt ctgtctctca aattctggaa ttaaagattt gcgccacttt tccccacttc
 1201 cacccecggc tgtgggagtg gactgggttg aagggtggaat tttttttttt tttttttttt
 1261 tttagtgaat aaagggggga ttggaaatat ccctactttc aactctagta tatttcagaa
 1321 accaagcctc agagatgtgc gtgctgctgt gtgtgtgtgt gtatgtgtgt gtgtctcaca
 1381 gcaagaaaca gattttatta tttatttttt atttatttat tttttgcaag tgactggcta
 1441 ggaagagtgg ggaagcggga ggacaaatgg ggaagaggga gcatttccgc aagtgtggtg
 1501 ctgaccaat cagcgcgcgc catgggggtat ttaaggctga gggcggctag gcctcggcac
 1561 ctaaccctga ttttcattag ctgtgggttc tggctttttg ttctccgccc gctgtttttc
 1621 tcgctgactt ccagcgggcc aggaaaagtcc agacctgcag cgggccaccg cgcgttcccg
 1681 agcctcaaaa acaaacgtca gcgcaggagc tccaggttcg ccgggagctc cgcggcgccg
 1741 ggccgcccag tcccgtaccc gcctacaggg cgcgcccgcc ctgggggtctt aggactccgc
 1801 tgccgcgcgc aagagctcgc ctctgtcagc cgcggggcgc cgggggctgg ggccaggccg
 1861 ggcgagcgcc gcgaaggaca ggaatggaac tgggtccccgt gttcgggtgtc ttacctgagc
 1921 tgtgggaagt gcacccggaa ctcggttctc acaacccccca ttcccgttgg ggaaatgccc
 1981 cgctgcaggg cgggcccgtc gaacctgcga cttctgggga aaggggcttc ggtgtgagac
 2041 ggtagccagc caaagggtat atatcgccct cagccccctg cccctccac ttttgtctaa
 2101 tactcctgtt tctgttgtgc agattttgca ggcgtttcgc tggctctgcc tgaacgagct
 2161 atgcagccat gtggtccttg ggggtggggg tggggatggg aggactacag gcgtagatct
 2221 tcatactggg tttgtgtagt gctgggaatt gaacctagtt tcctaagttc tctatcaact

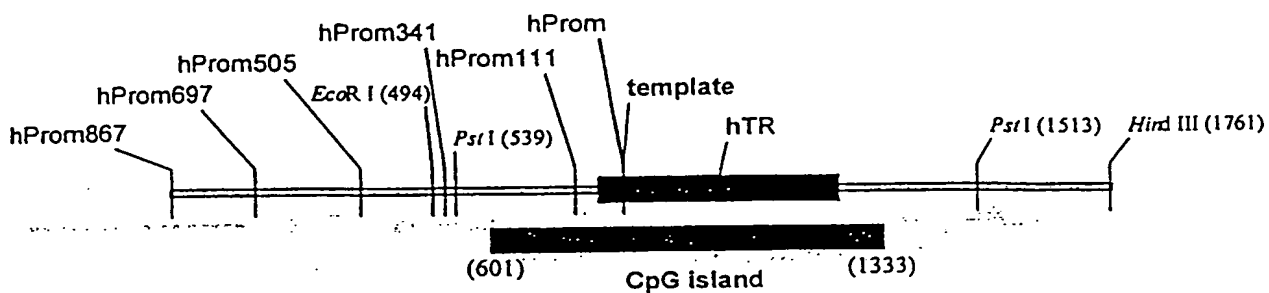
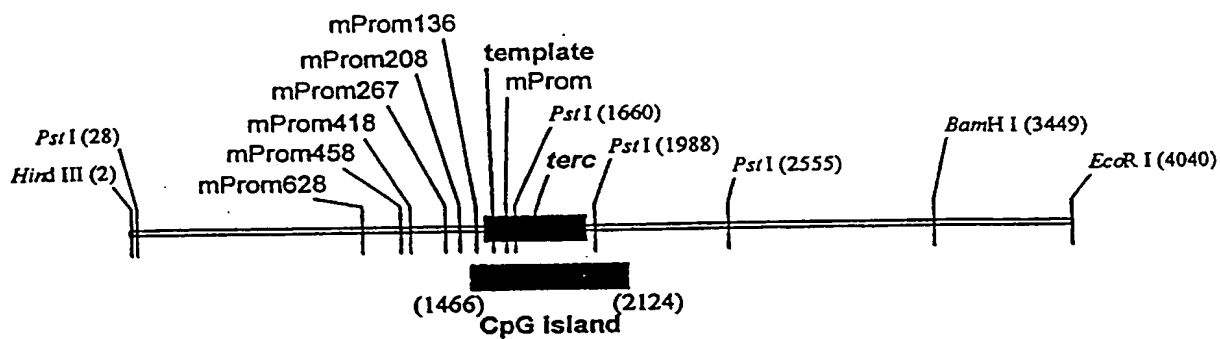
Fig. 2

3/29

2281 ggtattccca ttgtatggga gatttttttt ttcttttgt tatgggggcg ttgaacattt
 2341 tgtaaacaat tagaaaatct agtagtttt taatgaaaat gttcactttt ctttgtcttt
 2401 gggatgcaaa acattacatt gaagctgaga agtttaaaga tgcgtgtctt cccctgccta
 2461 ccttcgctgt cacacagaac ctgttatctt ttcagaaaag aaaatgagat aggcagggtg
 2521 gatctggagt ttcaaggccc ttgcctgggc tgcagagtga gttaggccac accagaaaag
 2581 tatgtgtcaa aaacaaagaa gaaaggcttt gtgggggggt gggtagcaaa cgatcttaat
 2641 cccctgtgct tgggaggccc gcaaggggga tatctgtgaa ggagacaaac aaagctacac
 2701 tgtgtggtaa acaaaaaacg aggaggagga gcaagaagaa tatgagagcc cacggaagga
 2761 agagtatcag tccccaggcc accagttcct caggggtaac tatgtttgtg agtgtctcgg
 2821 tgccttgact tcctcagtac ttttctgggt tttagtcata aaaaacattg aagagatgaa
 2881 gaagtgtatg tttagtaagt acataccaaa agtttgtgag ctatatgcat atagcaactc
 2941 agtcacctga aacaggcccc ttgcagctaa catatttctt agtattacta ttataaagac
 3001 taggggagtt tctaagccgg cactccttac aaggggacgaa gccatgttca gctccagctt
 3061 gccaaagatt tgaaacccaa cgtcaagcct gacgagttcg agcctggcat ctctcagccg
 3121 ctgctcgagc tggagatgac cacggatctc aaggcacagc tgtgggaact caacatcacc
 3181 gaagccaagg aaaattgaag ttggtggtgg tcagaaggct gttataattt ttgtaccagt
 3241 tcctcagctt aaatctttcc agaaaatcca agtctggcta gtttgtgaat tggagaaaaa
 3301 gttcagcggg aagcacgtgt cttcattgct cagaagagga tctgtccaag ccaaccagga
 3361 aaagctgtac gaaaaataag ccaaagcacc ctagaagctg caccctgaca gcagtgcattg
 3421 tcttctcaag tgaaattgtg ggaaagagga tccatccgtg tgaaactgga tggcaatctg
 3481 gagcaggttc atcttcctct ctggtacatc ccatgtctcc tcatctccat cctccctct
 3541 gcctctgtgt ctcatctcta aaactctcag cccatcttcc tttaccactg cccaatcaca
 3601 ggctctagcc ttacctttca cctgccctca cctgcttata gacagcaatc tacatttctc
 3661 cctttttgtc caattaaaag actcttttct ctcggtatata aaatgagcac aactattatt
 3721 accattctgt aatttataaa gtatagatag acctaacacc cagtctatca ttttgacagt
 3781 taaataaagc attctgcaat cctatcctaa ctttaaaagg cttataattc tacacttggt
 3841 atgtcctggt tcagcttgta tattagaaaa ccatctcaaa ttatatatat atatataatta
 3901 cacacacaca tatgtatata tacatatata tgtatacaca cacacacata tatatatgta
 3961 tatgtatgta tgtatgtata tatatatact tttaatgcta aatagcctgg gttggctaag
 4021 actacttcaa tcctgccaga attc

Fig. 2 (cont)

4/29

*Fig. 3A**Fig. 3B*

09601267.082500

5/29

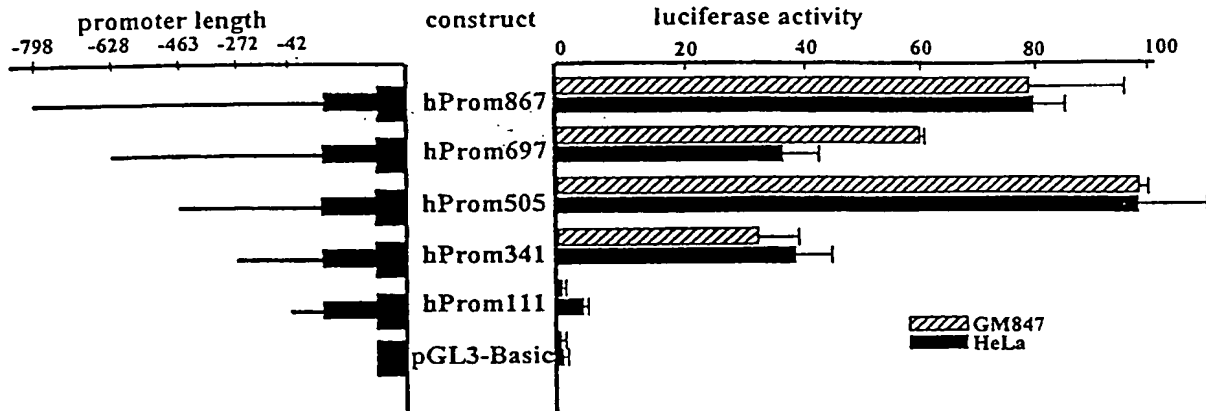
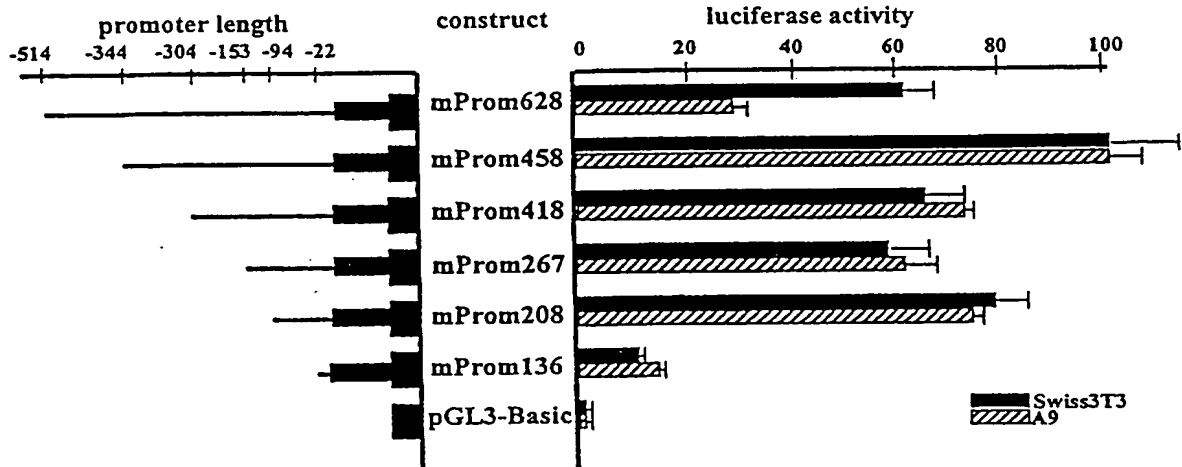
-798 ^{hProm867} agctactcaggaggctgagacacgagaatcgcttgaacccgggaggcaga
 -748 ggttgacagt^{Zeste}gagccgagatcacgccactagactccatccagcctgggcca
 -698 aagagcaagactccgtctcaaaaaaaaaaatcgttacaatttatggtgga
 -648 ^{hProm697} ttactccctcttttttacctcatcaagacacagcactactttaagcaaa
 GR
 -598 gtcaatgattgaaacgcctttctttccctaataaaaggagattcagtcct
 cMYB NF1 PEA3
 -548 taagattaataatgtagtagttacacttgattaaagccatcctctgctca
 AFP1/BRN2
 -498 aggagaagctggagaaggcattctaaggaaaaaggggcagggttggaact
 PEA3/c-Ets-2 Spl/NF-E2 cMYB
 -448 ^{hProm505} cggacgcacccactgagccgagacaagattctgctgtagtcagtgctgc
 Zeste GCN4/AP1
 -398 ctgggaatctattttcacaaagttctccaaaaaatgtgatgatcaaaact
 myogenin GR GR
 -348 aggaattagtggttctgtgtcttaggccctaaaaatcttctgtgaattcca
 GR/PR/AR F2F/Pit-1a Pit-1a
 -298 ^{hProm341} tttttaaggtagtcgaggtgaaccggtctgtggtctgcagaggatagaaaa
 GATA-1
 -248 aaggccctctgatacctcaagttagtttcacctttaagaagggtcggaag
 E1A-F
 -198 taaagacgcaaagcctttcccgacgtgcggaagggcaacgtccttcctc
 NF1 PEA3 PU.1
 -148 atggccggaaatggaactttaatttcccggttccccccaaccagcccgccc
 Spl
 -98 gagagagtgaactctcacgagagccgcgagagtcagcttgccaatccgtg
 API GCN4/AP1 CCAAT Box
 -48 ^{hProm111} cggtcggtcggtccctttataagccgactcgcccggcagcgcaccgg
 PEA2/PEBP2 GAGA TBP/TFIID
 gttgcggagggtgggcctgggaggggtggtggccattttttgtctaacc
 template
 taactgagaagggcgta^{hProm}

Fig. 4A

09601267-082500

Fig. 4B

7/29

*Fig. 5A**Fig. 5B*

8/29

Oligo's Used: human

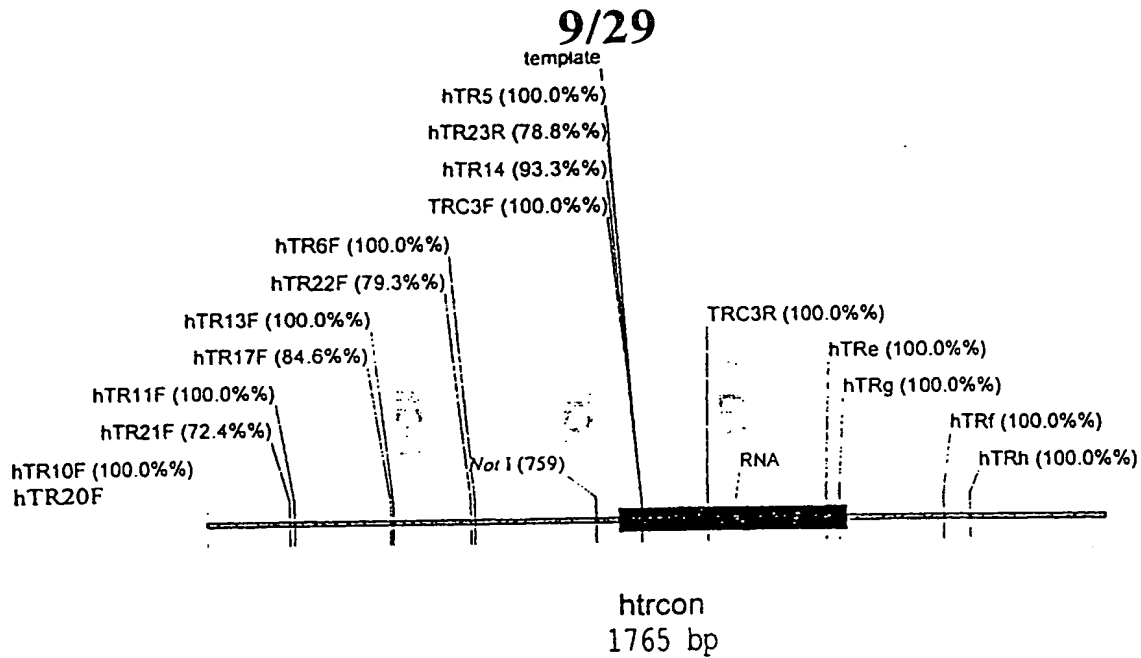
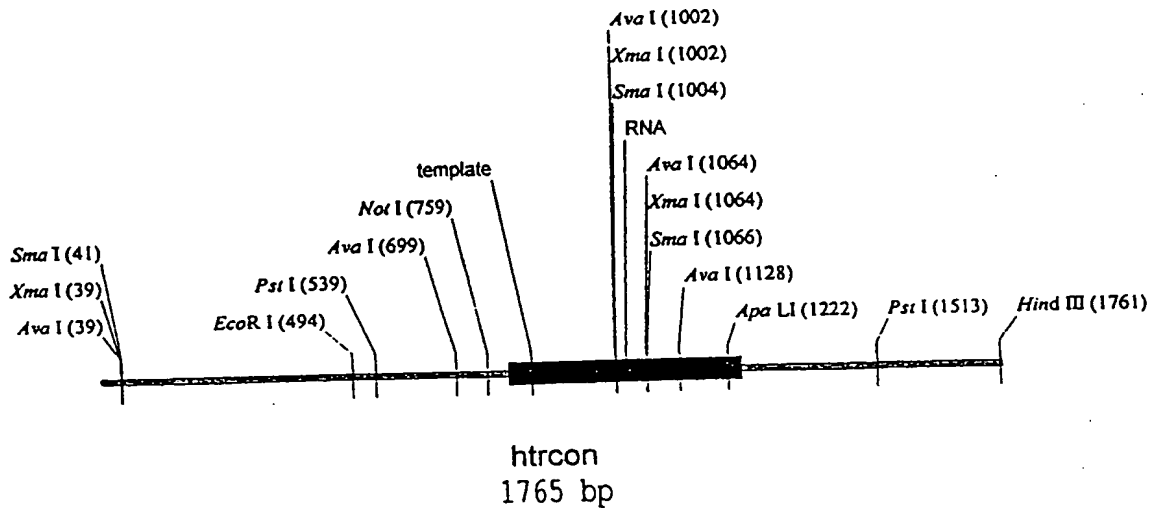
Name	Sequence	Comments
hTR5	TACGCCCTTCTCAGTTAGGGTTAG	
hTR14	GGATCCTACGCCCTTCTCAGTTAGGGTTAG	hTR5 with BamHI site
hTR13F	ACTGAGCCGAGACAAGATTC	
hTR17F	GGATCCACTGAGCCGAGACAAGATTC	hTR13F with BamHI site
hTR10F	AGCTACTCAGGAGGCTGAGA	
hTR20F	GCGCTCGAGAGCTACTCAGGAGGCTGAGA	hTR10F with XhoI site plus gcg clamp
hTR11F	CATCAAGACACAGCACTACT	
hTR21F	GCGCTCGAGCATCAAGACACAGCACTACT	hTR11F with XhoI site plus gcg clamp
hTR6F	GTCTGGTCTGCAGAGGATAG	
hTR22F	GCGCTCGAGGTCTGGTCTGCAGAGGATAG	hTR6F with Xho site plus gcg clamp
hTR5	TACGCCCTTCTCAGTTAGGGTTAG	
hTR23R	CGCAAGCTTTACGCCCTTCTCAGTTAGGGTTAG	hTR5 with HindIII site plus cgc clamp
hTRe	CTGAGCTGTGGGACGTGCAC	
hTRf	AGACGGGAGAACCCACGCAG	
hTRg	CTCGGCTCACACATGCAGTT	
hTRh	TCTGCAGAGCAGGAAGTAAGT	
TRC3F	CTAACCCCTAACTGAGAAGGGCGTA	
TRC3R	GGCGAACGGGCCAGCAGCTGACATT	

Oligo's Used: Mouse

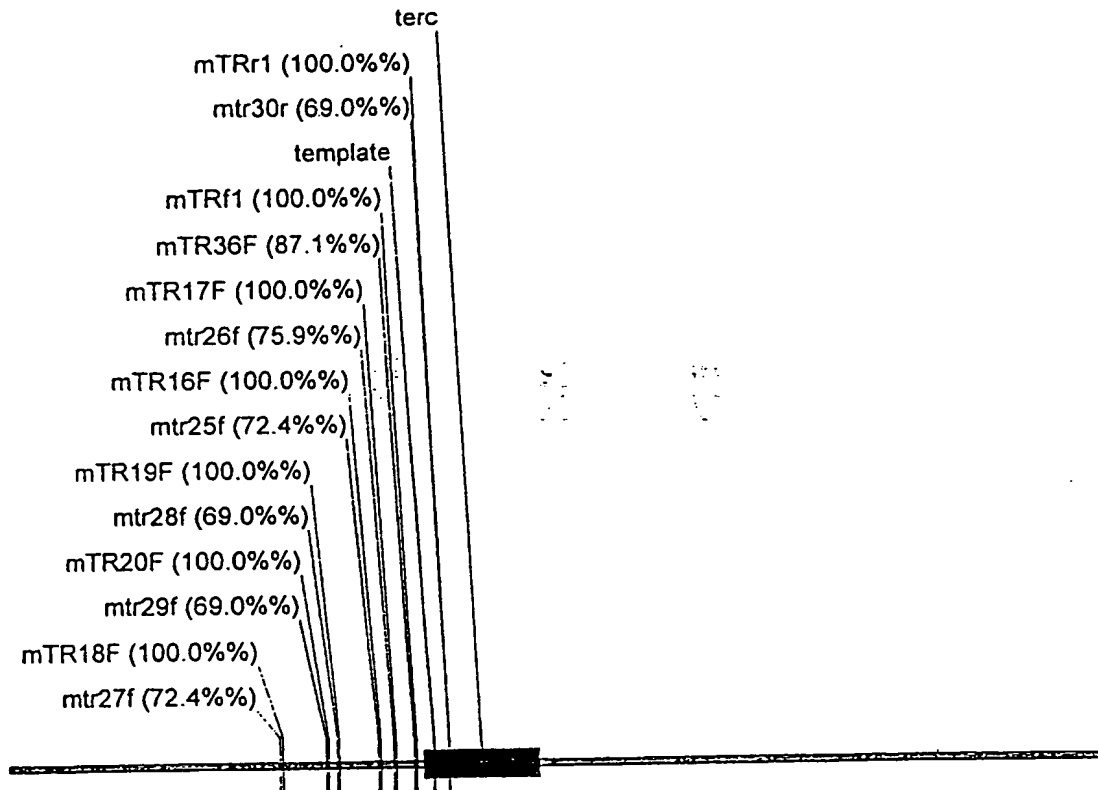
Name	Sequence	Comments
mTR16F	GTGTCTCACAGCAAGAAACA	
mtr25f	GCGCTCGAGGTGTCTCACAGCAAGAAACA	This is mtr16f with XhoI site plus gcg clamp
mTR17F	GTGACTGGCTAGGAAGAGTG	
mtr26f	GCGCTCGAGGTGACTGGCTAGGAAGAGTG	This is mtr17f with XhoI site plus gcg clamp
mTR18F	TGTGACCTTGAAGTACAGAC	
mtr27f	GCGCTCGAGTGTGACCTTGAAGTACAGAC	This is mtr18f with XhoI site plus gcg clamp
mTR19F	GGACTGGGTTGAAGGTGGAA	
mtr28f	GCGCTCGAGGGACTGGGTGAAGGTGGAA	This is mtr19f with XhoI site plus gcg clamp
mTR20F	TGCGCCACTTTTCCCCACTT	
mtr29f	GCGCTCGAGTGCGCCACTTTTCCCCACTT	This is mtr20f with XhoI site plus gcg clamp
mTRr1	CCGCTGGAAGTCAGCGAGAA	
mtr30r	CGCAAGCTTCCGCTGGAAGTCAGCGAGAA	This is mTRr1 with HindIII site plus cgc clamp
mTR36F	GCGCTCGAGTCGACCAATCAGCGCGGCCAT	This is Xho I site PCR primer plus gcg clamp
mTRr1	CCGCTGGAAGTCAGCGAGAA	
mTRf1	TCGACCAATCAGCGCGGCCAT	

Fig. 6

09601267-032500

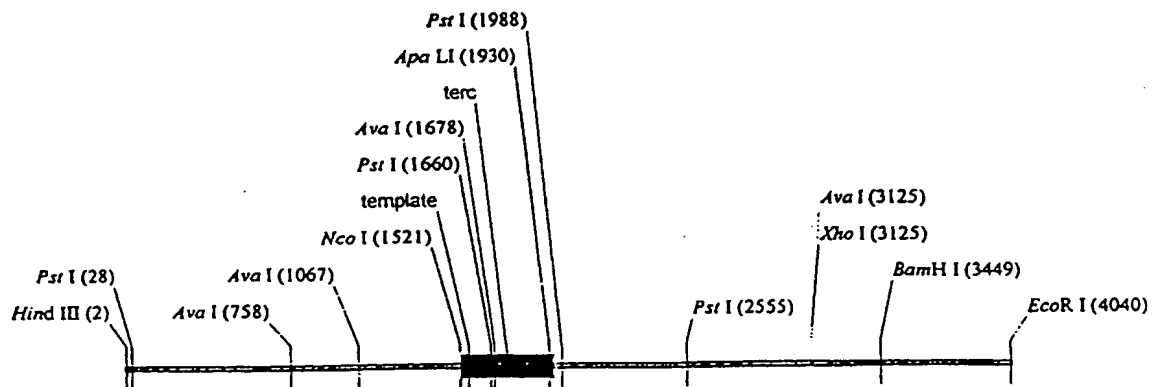
**Fig. 7A****Fig. 7B**

10/29



tercCon
4044 bp

Fig. 8A



tercCon
4044 bp

Fig. 8B

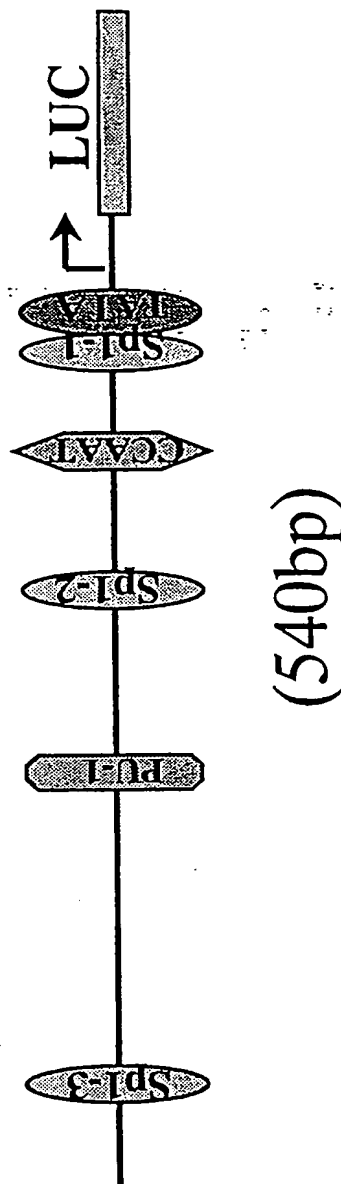
09601267 082500

11/29

TTGTGACCTTGAACACAGACCTCCTGCTCAGCCTCCTACAAGCTGGGATTATAGGCTCGGCTCAGCTACCCCTTGAAA
TTCTTTTCTTCTGGAACACAGTACCTGGTTGGCCATGCACCTCACAAAGAGATCCGCCCTGCCCTTCTGTCAAATTTCTGGA
ATTAAAGATTTCGCCACCTTTTCCCCACTTCCACCCCGGCTGTGGAGTGGACTGGGTGAAGGTGGAATTTTCTTTT
TTTCTTTTATTAGTGAAAAAAGGGGGATTGGAAATATCCCTACTTCAACTCTAGTATATTTCAGAAACCAAGCCTCAG
AAATGTGCGTGCGTGCTGT
TTTATTATTATTTTTCAAAGTGACTGGCTAGGAAGAGTGGGGAAGCGGGAGGACAAATGGGGAAGAGGGAGCATTTCCGC
AAGTGTGGCTCGACCAATCAGCGCGCGCCATGGGGTATTAAAGTTCGAGGCGGGCTAGGCCCTCGGCACCTAACCCCTG
ATTTTCATTAGCTGGGTCTGGTCTTTCTGTTCTCGCCCCGCTGTTTTTCTCGCTGACTTCCAGCGGA

Fig. 9

12/29



<i>Human Promoter</i>
TATA
CCAAT
SP-1
AP1
PEA2/PEBP2
PU.1
PEA3
GATA1
C-MYB
GR/PR/AR
F2F
ZESTE

Fig. 10

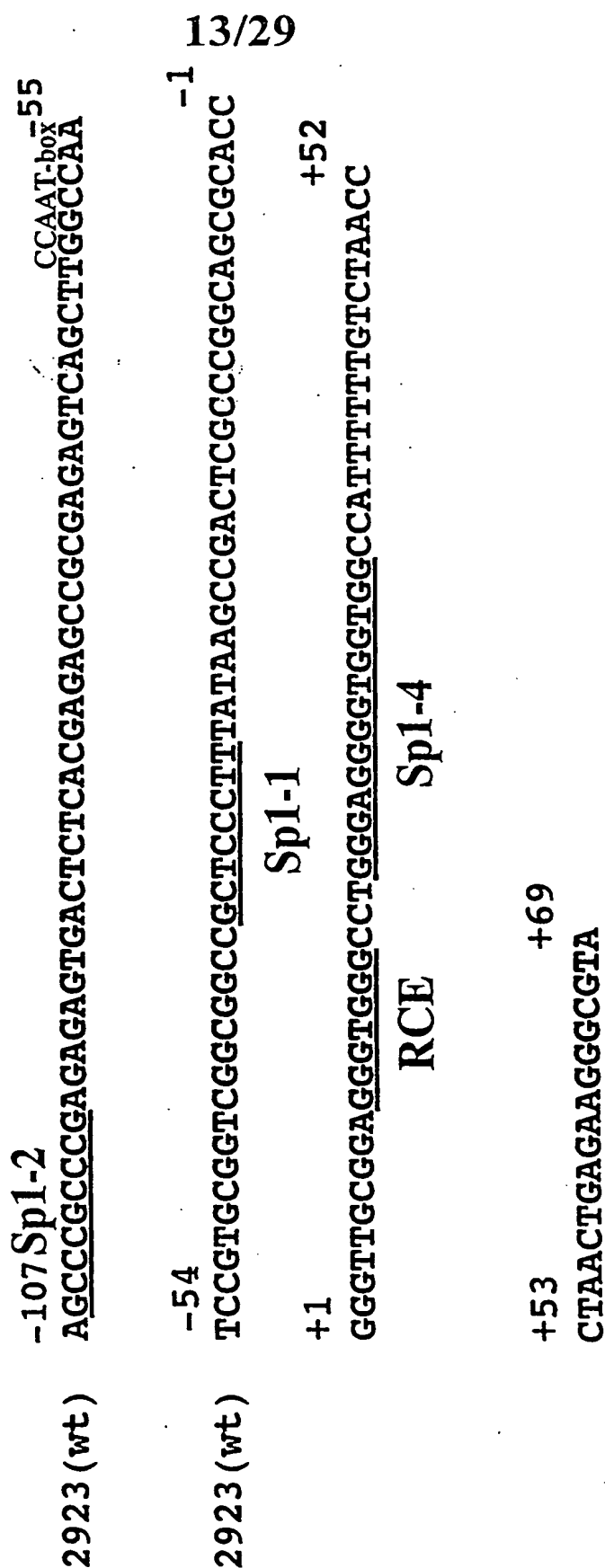


Fig. 11

14/29

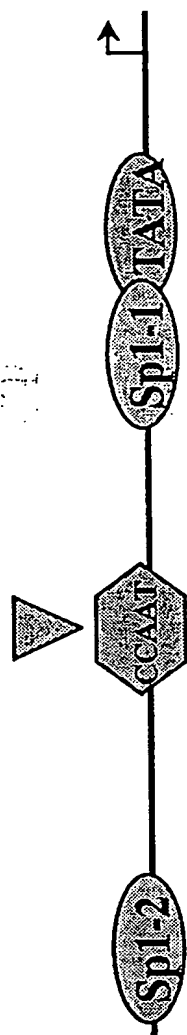
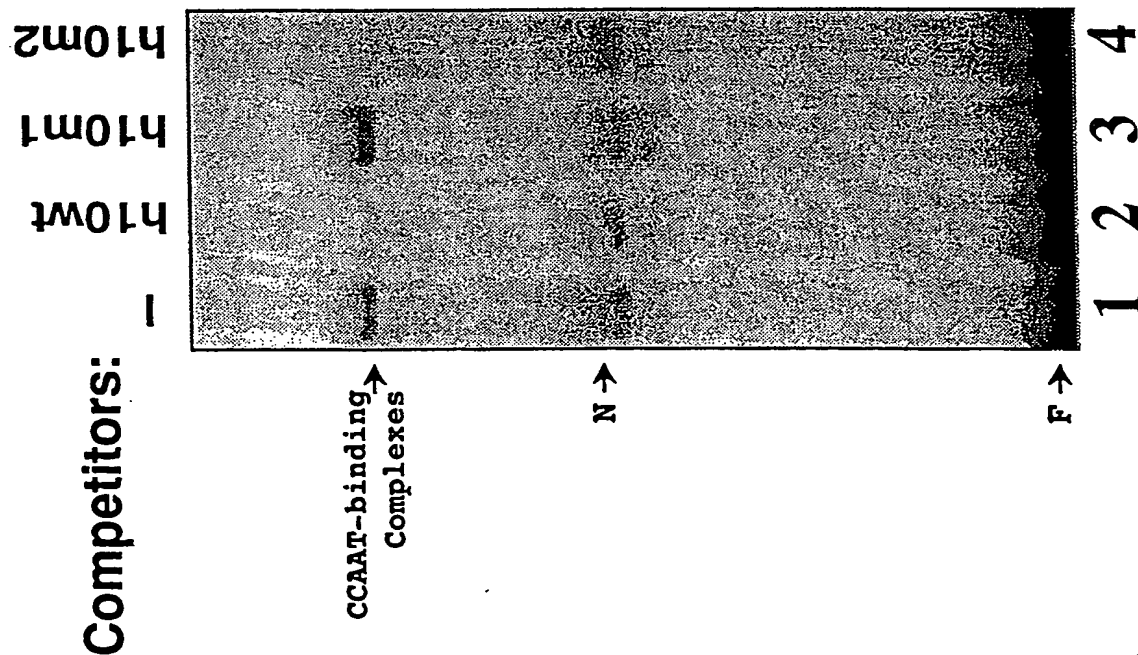
Oligonucleotide	Position	Sequence*	Purpose
h11 ^a	-2 to +36	CCGGTTGCGAGGGTGGGCTGGAGGGGTGGTGCC	RCE(+12,+16 and +30, +34) and Sp1.4 binding site
h111 ^a	"	CCGGTTGCGGAAATGGGCTGGAGGGGTGGTGCC	RCE1 mutation from ggg to aaa (+11/+13,)
h112 ^a	"	CCGGTTGCGGAGGGTGGGCTGGGTAAGGTGGTGCC	Sp1.4 mutation from agg to taa (+24/+26)
h113 ^a	"	CCGGTTGCGGAAATGGGCTGGGTAAGGTGGTGCC	Mutant of both RCE1 and Sp1.4 binding site(+11/+13, +24/+26)
h11c ^a	-2 to +23	CCGGTTGCGGAGGGTGGGCTGGG	RCE1 binding site
h11d ^a	+14 to +36	GCCTGGGAGGGTGGTGCC	Sp1.4 or RCE2 binding site
h111a ^{a,b}	"	CCGGTTGCGGAAATGGGCTGGG	RCE1 mutation from ggg to aaa(+11/+13)
h112b ^{a,b}	+15 to +36	GGGCTGGGTAAGGTGGTGCC	Sp1.4 mutation from agg to taa
h112C ^{a,b}	"	GGGCTGGGTAAGGTAATGGCC	Sp1.4 and RCE2 mutant from aggggtgg to taaggtaa(+24/+26, +30/+31)
h11e ^a	"	GGGCTGGGAGGGGTAATGGCC	RCE2 mutant from gg to (+30/+31)
h10 ^a	-63 to -42	CTTGCCCAATCCCTTATAGCCGACT	h10 bootprinting region containing CCAAT binding site
h101 ^a	-63 to -42	CTTGAGTCTCCCTTATAGCC	CCAAT motif mutation from ccaa to agtc, (-58/-55)
h10m11 ^b	-74 to -45	GCGAGATCAGCTTGGAGTCTCCGTCGG	CCAAT motif mutation from ccaa to agtc, (-58/-55)
h10m2 ^{a,b}	-63 to -42	CTTGCCCAATCCCTTATAGCCGACT	h10 mutation from gtgc to tgal, (-51/-47)
h9 ^a	-44 to -17	CGGCGCCGCTCCCTTATAGCCGACT	h9 bootprinting region containing SP1.1 binding site
h91 ^a	-44 to -21	CTTAGCCGCTCCCTTATAGCC	h9 mutation from ggog to ttac, (-43/-40)
h910 ^b	-53 to -29	CCGTGCGGCTTACGCGCTCCC	h9 mutation from ggog to ttac, (-43/-40)
h911 ^a	-44 to -21	CGGCTAAACTCCCTTATAGCC	h9 mutation from ggog to taaa, (-39/-36)
h92 ^a	-44 to -21	CGGCGCCATAGCTTATAGCC	h9 mutation from gctc to alag, (-36/-33)
h921 ^a	-44 to -21	CGGCGCCGCTCATGCTATAGCC	h9 mutation from cctt to algc, (-32/-29)
h93 ^a	-44 to -21	CGGCGCCGCTCCCTTCGACGACC	h9 mutation from tata to cgac, (-28/-25)
h930 ^b	-38 to -14	CCGCTCCCTTCGACGCGGACTCGC	h9 mutation from tata to cgac, (-28/-25)
h4 ^a	-110 to -91	ACCAGCCGCCCGAGAGAGT	h4 bootprinting region containing SP1.2 binding site
h41m ^a	-110 to -91	ACCAGCCCGAACGAGAGAGT	SP1.2 mutation from cc to aa, (-101/-100)
h5 ^a	-471 to -452	GAAGAGGGCGAGGGTTGGA	SP1.3 binding site
h5m ^a	-471 to -452	GAAGAGGTTTCAGGGTTGGA	SP1.3 mutation from gg to tt, (-463/-462)

* Nucleotides corresponding to promoter sequences are given in uppercase letters from 5' end to 3' end; specific nucleotides mentioned in Purpose column are underlined. Highlight indicate mutagenesis nucleotide.

^a Complementary lowerstrand sequence for EMSA not shown.

^b For PCR-directed in vitro mutagenesis, complementary lowstrand sequence not shown.

Fig. 12



h10 (wt:-63 -42)	CTTGGCCAATCCGTGCGGTCGG
h10m1 (-58 -55)	:::::AGTC::::::::::
h10m2 (-51 -47)	::::::::::TGAT:::::

Fig. 13

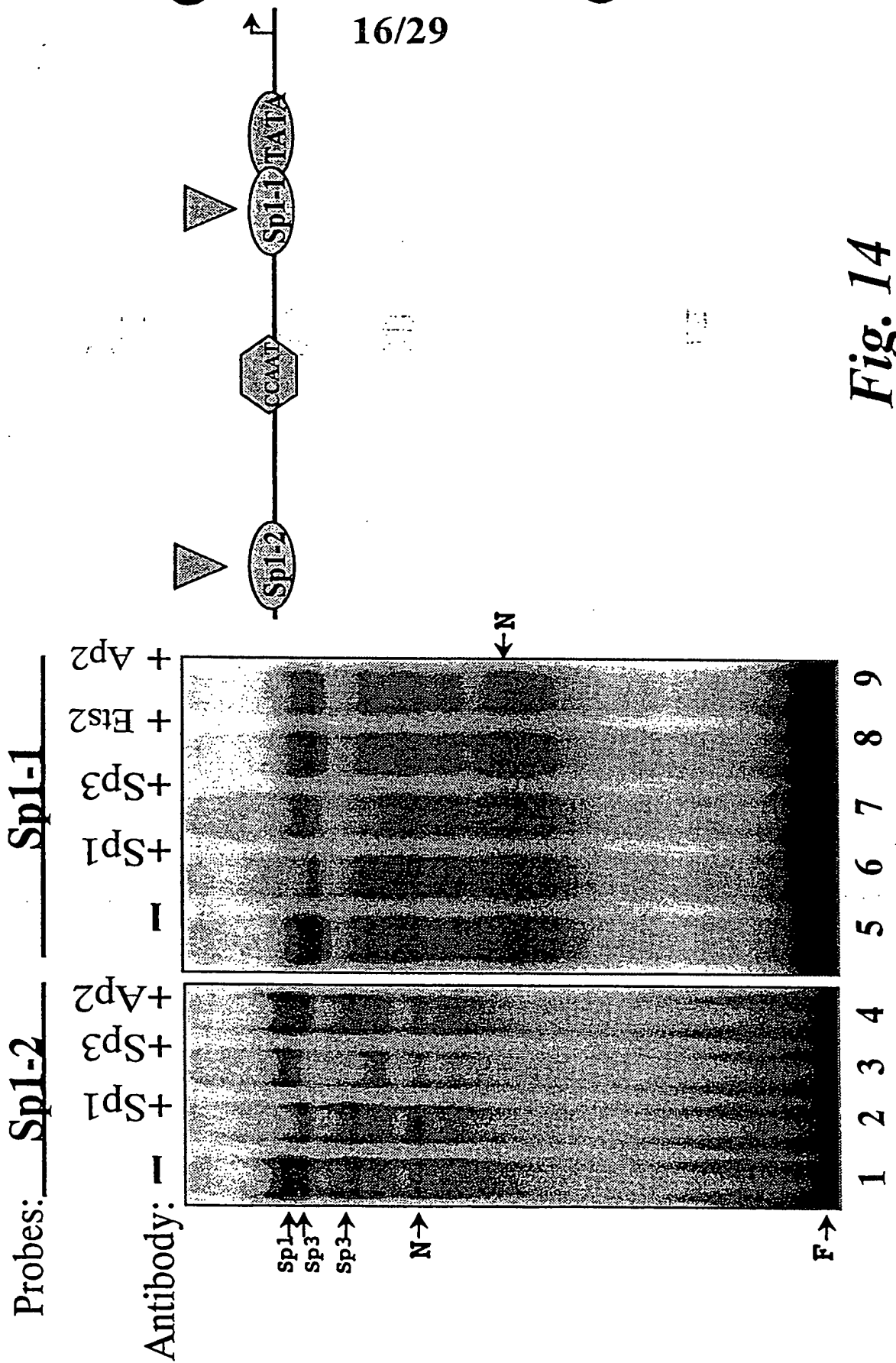


Fig. 14

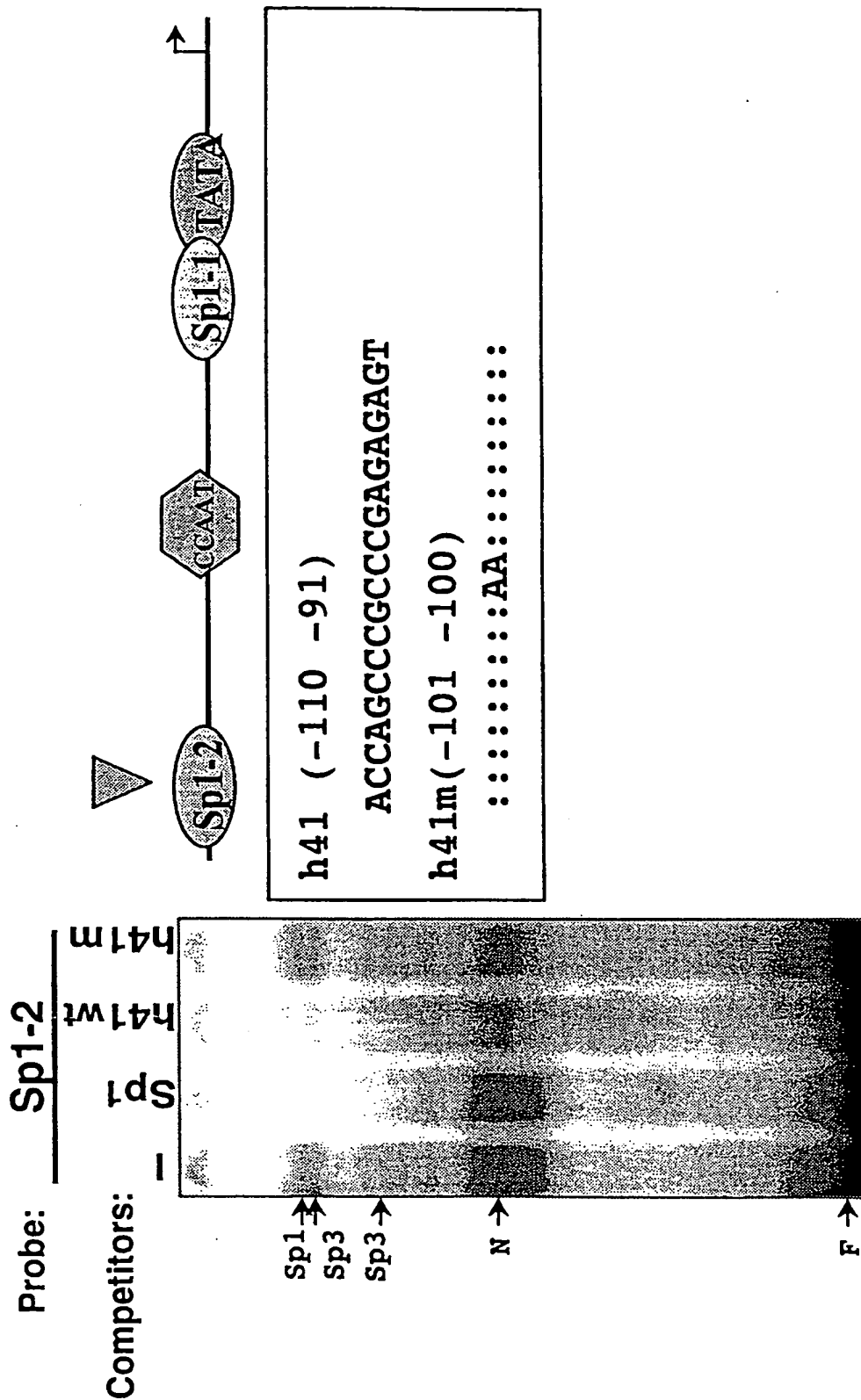


Fig. 15

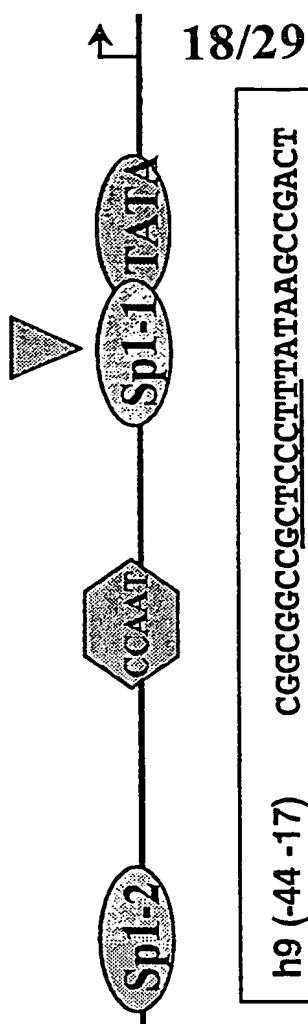
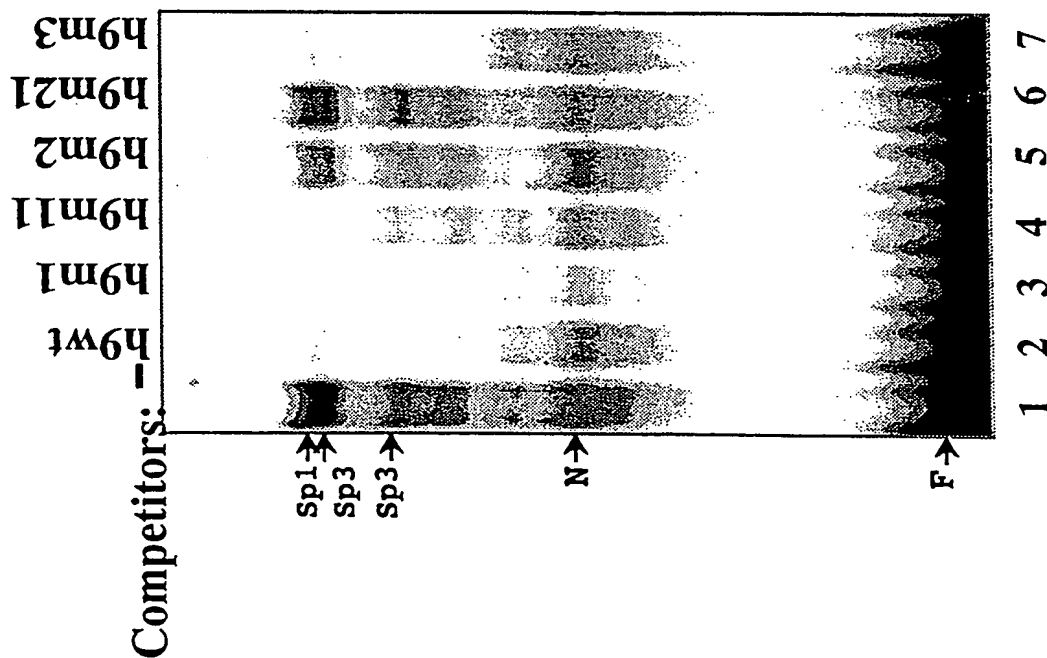


Fig. 16

19/29



- Gel shift assays identify DNA/protein binding activity
- Do mutation of these binding sites influence the promoter activity?

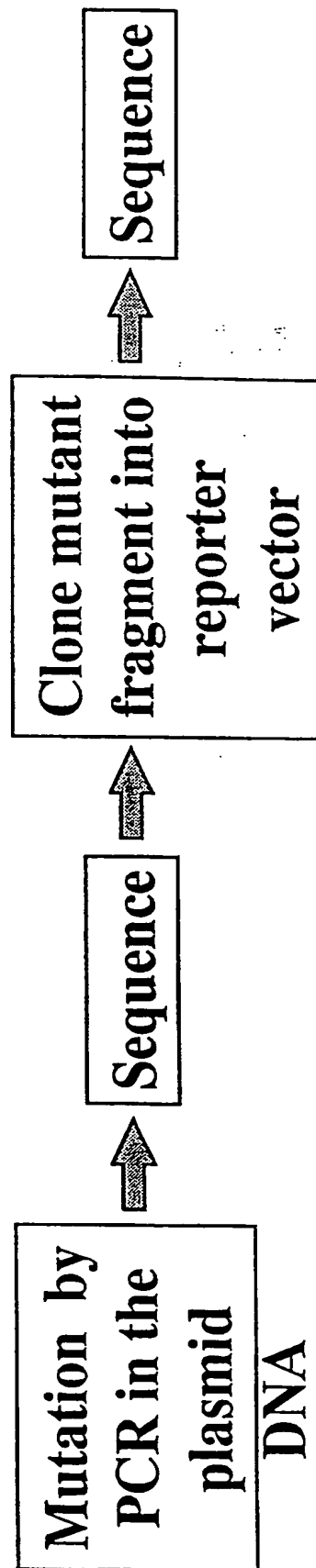


Fig. 17

20/29

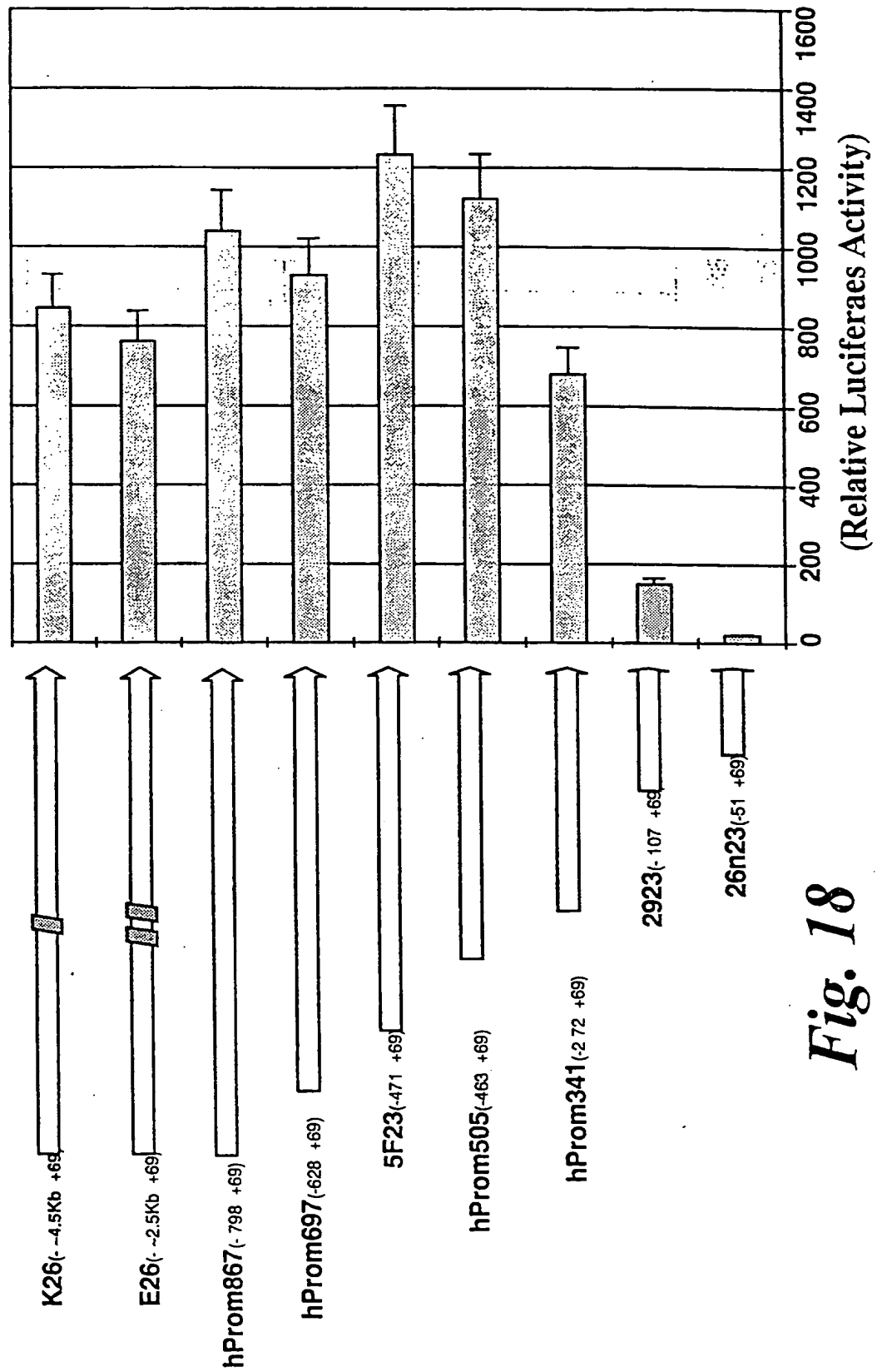


Fig. 18

21/29

2923(-107 +69) -107
 29m23(mSp1.2) -55
 1011
 29m292(mSp1-1,2)
 29m921(mSp1-1,2)

2923(-107 +69) -54
 102 -1
 910
 911
 92(mSp1.1)
 29m292(mSp1-1,2)
 921(mSp1.1)
 29m921(mSp1-1,2)
 930(mTATA)
 26n23(-51 +69)

26n23(-51 +69) +1
 29111(mRCE) +53
 29112(mSp1.4)
 111(Sp1.4)*
 112(RCE)*
 113(mSp1+mRCE)*
 115*
 114*

+54 +69
 CTAAGTGAAGGGCGTA

* Double mutagenesis by using pLh29m292 as template.

Fig. 19

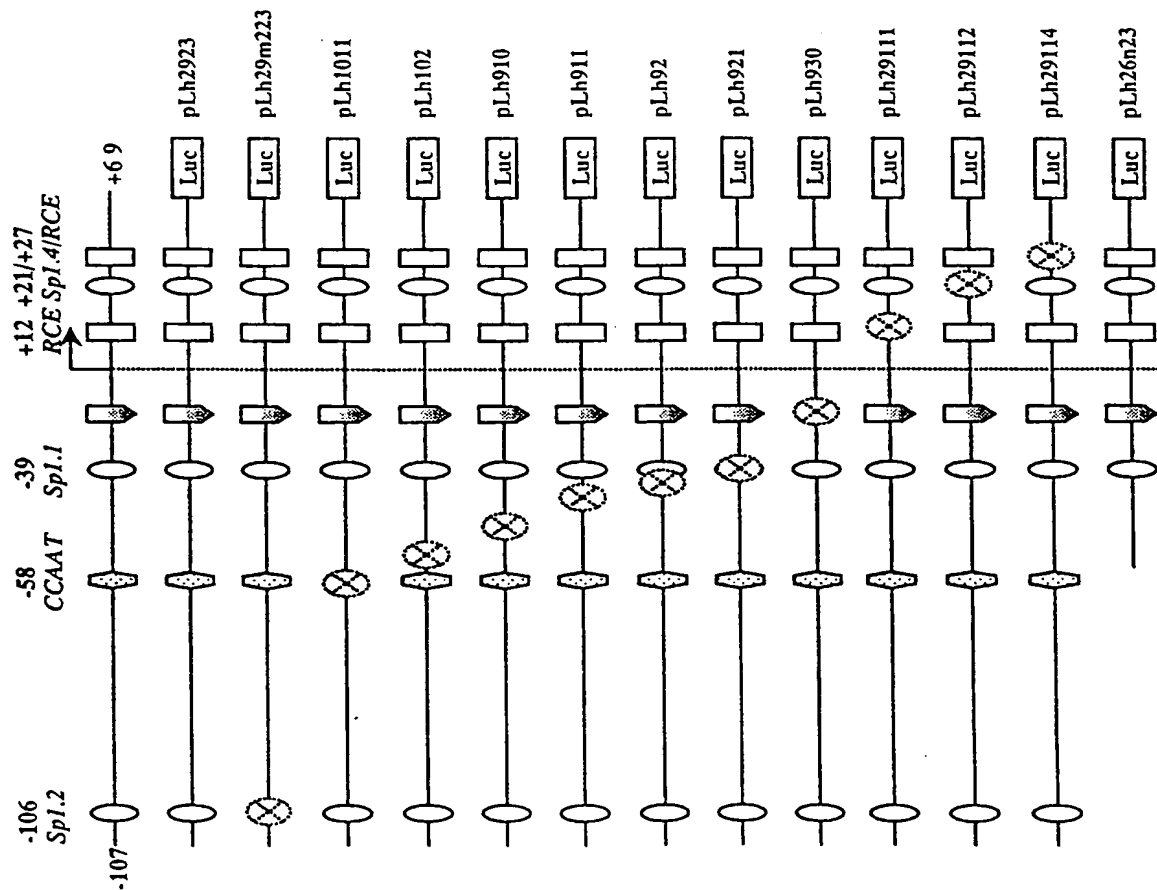
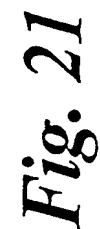
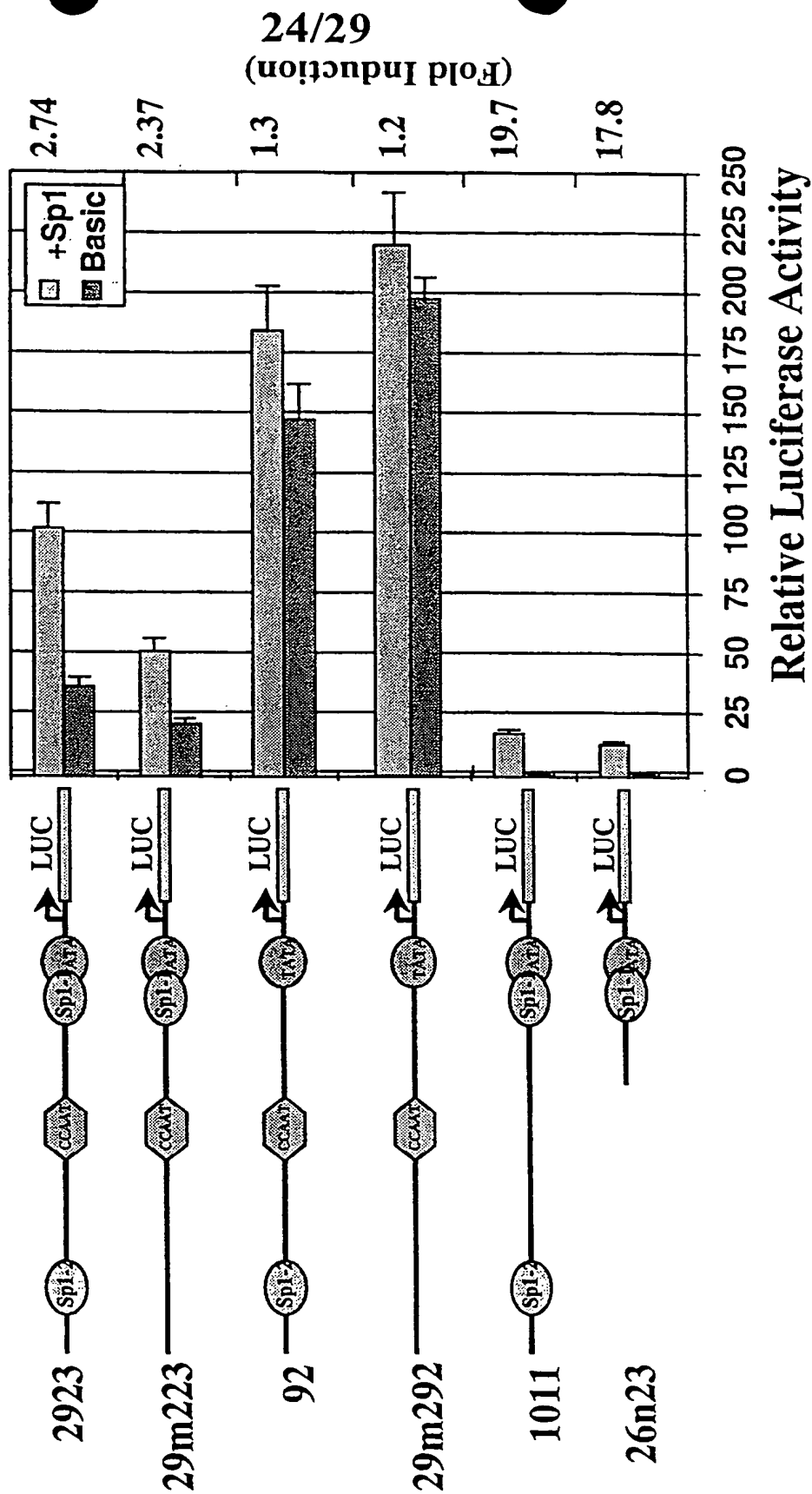


Fig. 20





25/29

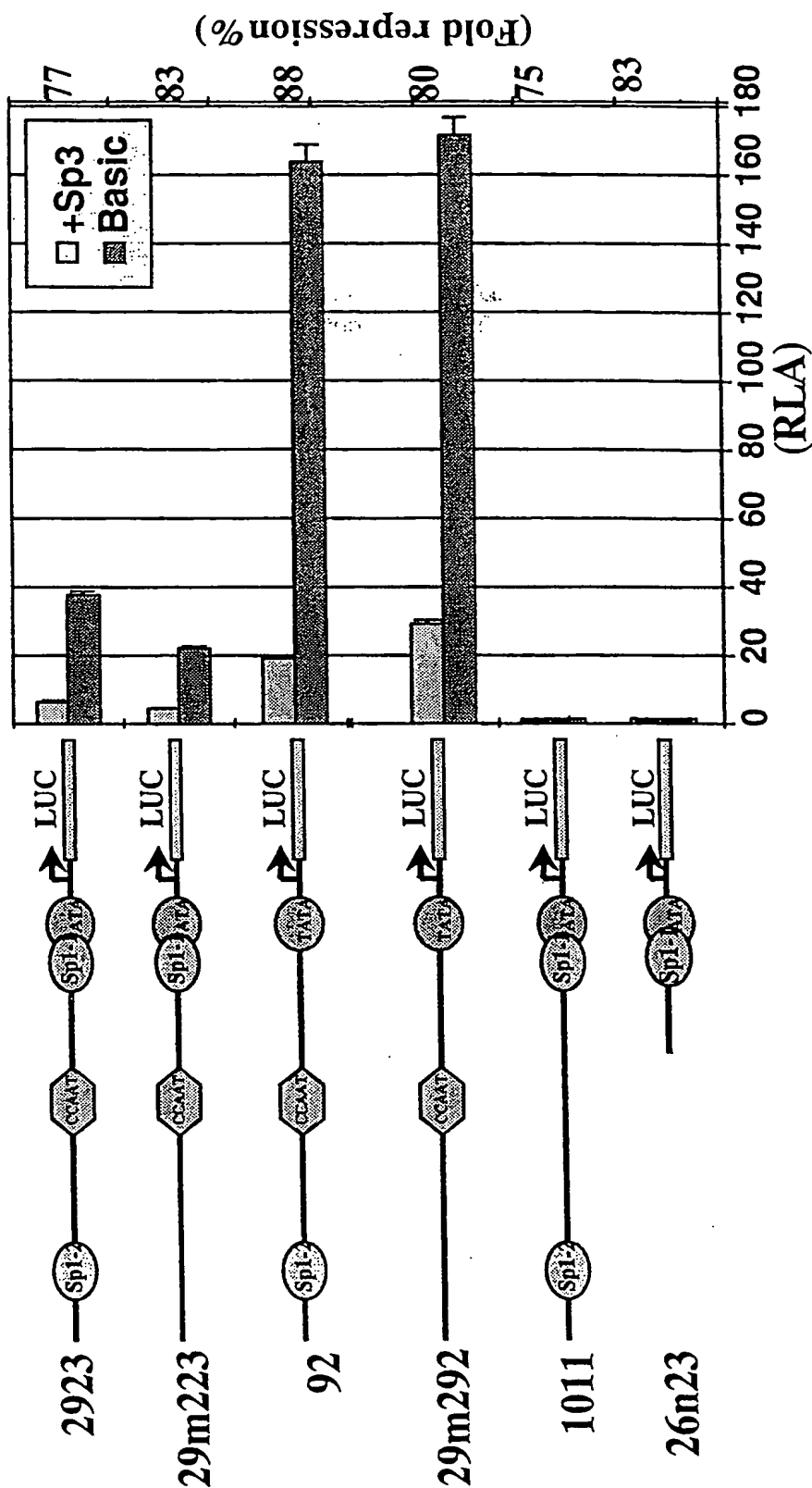
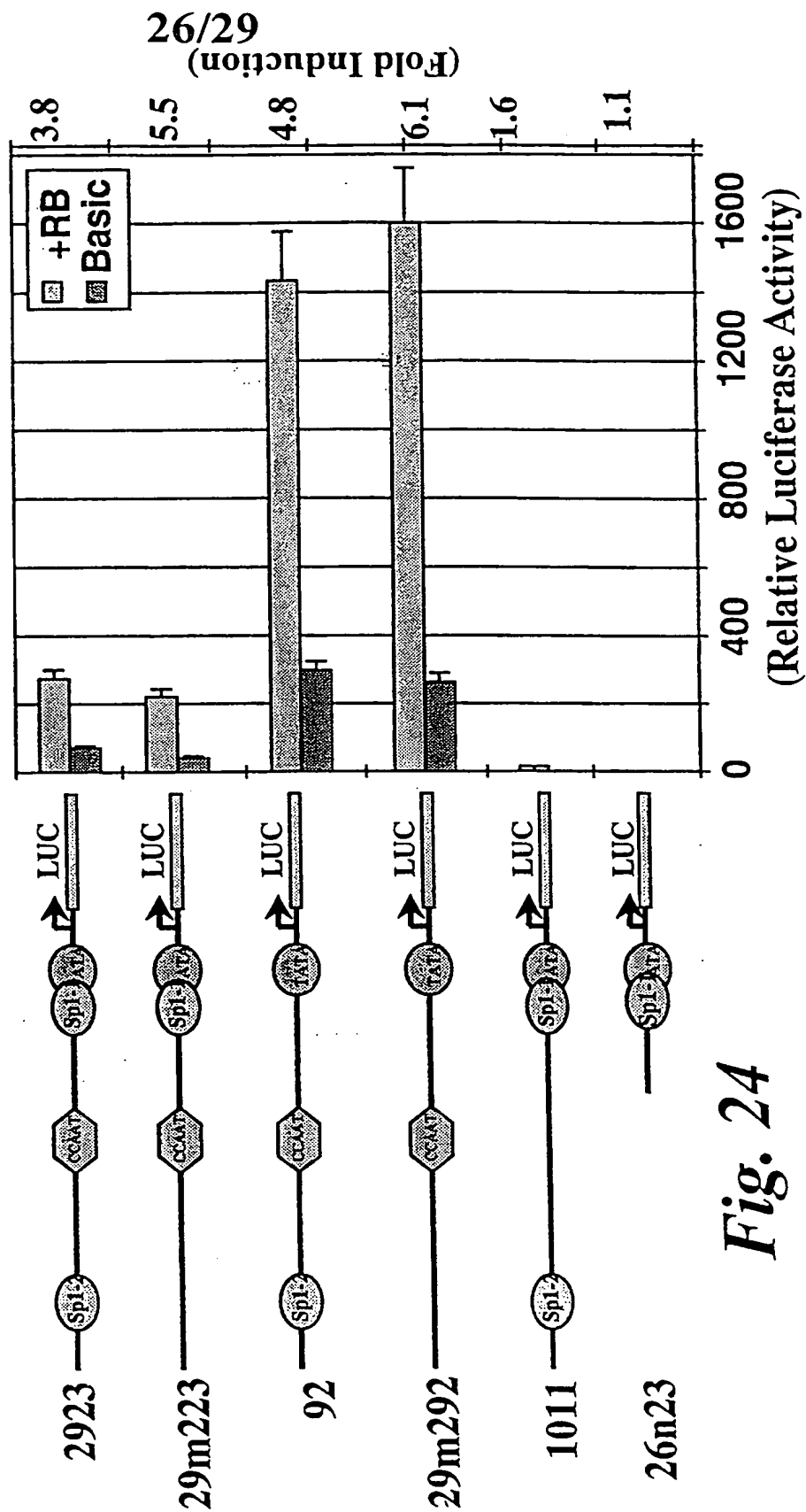
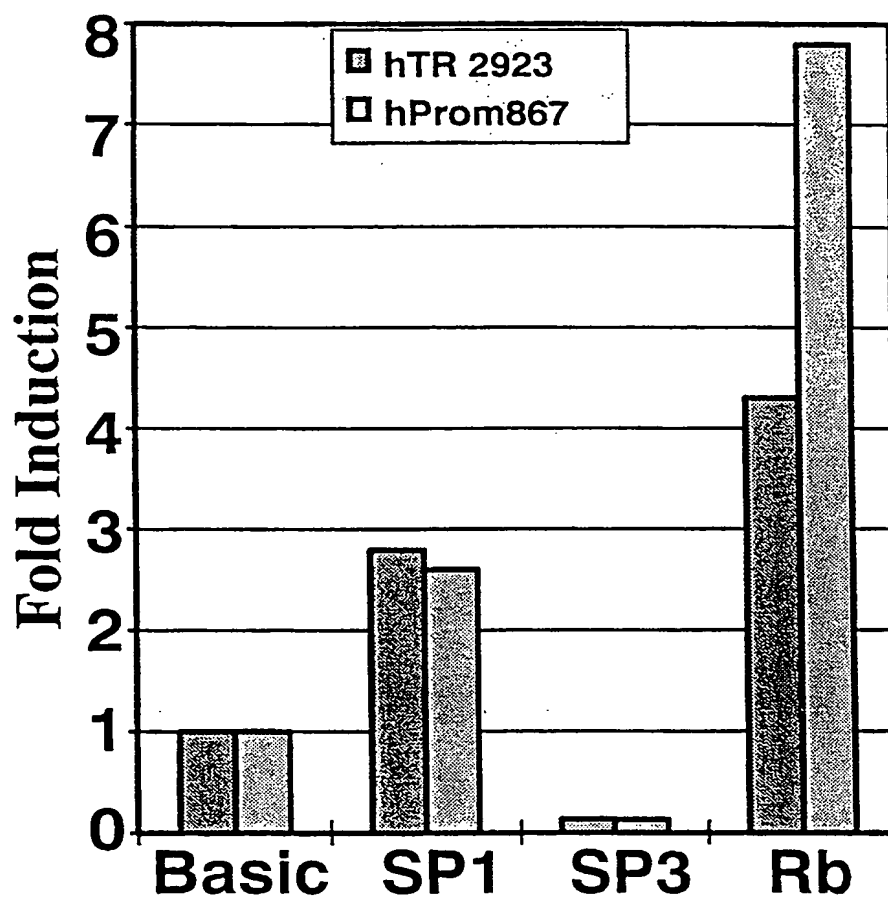


Fig. 23

SCANNED, # 14



27/29

*Fig. 25*

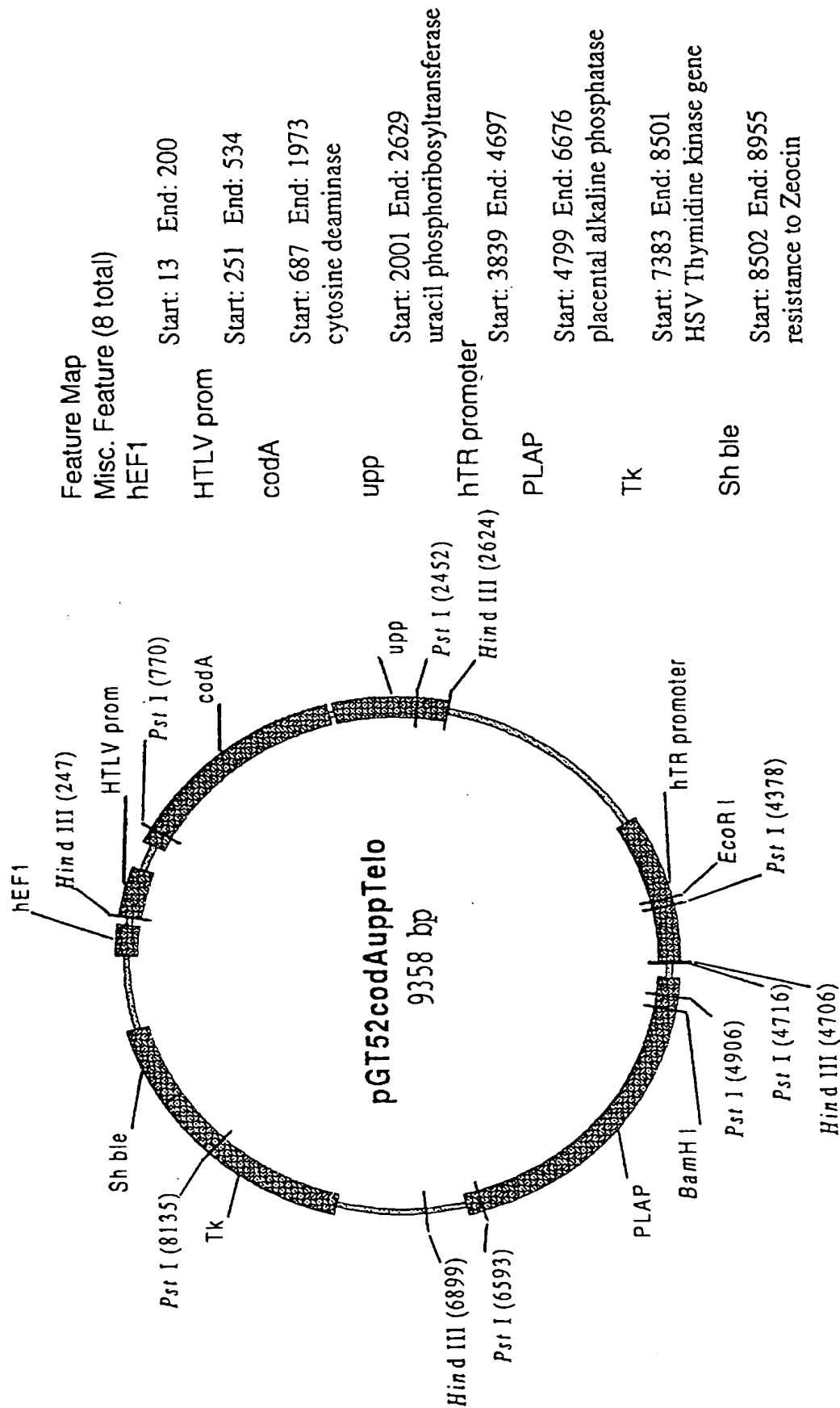


Fig. 26

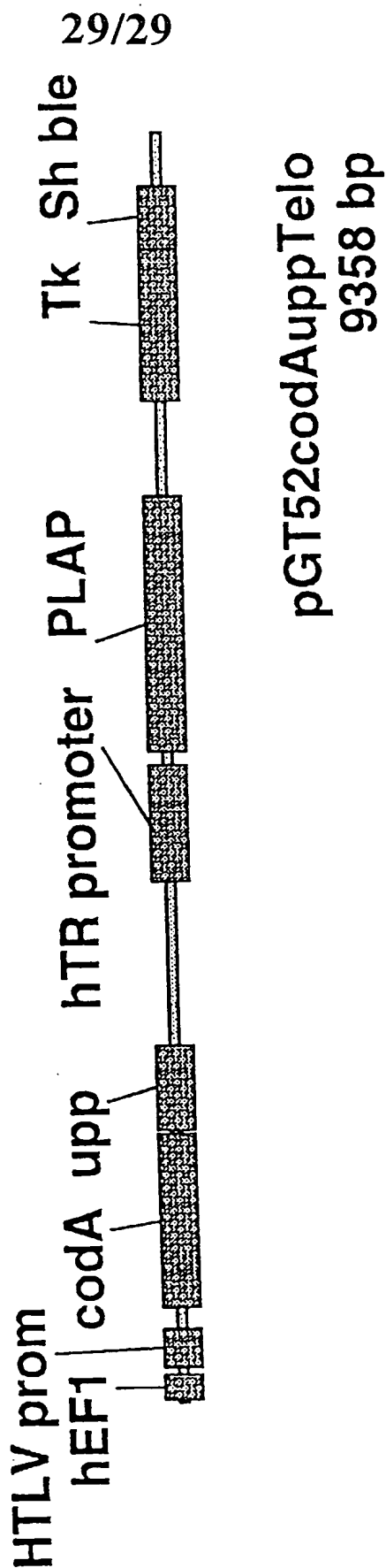


Fig. 27